# Thermal Inactivation and Conformational Lock of Bovine Carbonic Anhydrase

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**Abstract:** The kinetics of thermal inactivation of bovine carbonic anhydrase (BCA) was studied in a 50 mM Tris-HCl buffer, pH 7.8 using *p*-nitrophenyl acetate as substrate in absorbance of 400 nm by UV-VIS spectrophotometry. The number of conformational locks and inter-subunit amino acid residues of BCA were obtained by thermal inactivation analysis. The cleavage bonds between dimers of BCA during thermal dissociation and type of interactions between specific amino acid residues were also detected. The thermal inactivation curves were plotted in temperatures ranging between 40-70˚C. It was shown several phases for inactivation of BCA at 65˚C. Analyses of the curves were done by the conformational lock theory. The subunits are dissociated and several intermediates appear during inactivation through increasing the temperature in comparison with native state. Dynamic light scattering measurements was done to study the changes in hydrodynamic radius during thermal inactivation. Three distinct zones were shown in DLS data. Biochemical computation using ligplot is performed to find the inter-subunit amino acid residues for BCA.

**Keywords**: Carbonic anhydrase, kinetics, conformational lock, thermal inactivation, intersubunit interactions.

## 1. INTRODUCTION

Carbonic anhydrase (CA; carbonate hydro-lyase, EC 4.2.1.1) as a member of zinc-containing family enzymes catalyzes reversible hydration of carbon dioxide based on following equation [1, 2]

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

This enzyme is an appropriate target for a number of drugs, such as acetazolamide, methazolamide, dichlorphenamide and their aromatic derivatives [3, 4]. There are three BCA families which are evolutionarily unassociated, named alpha, beta and gamma [5]. Animal BCAs are alpha type. There are seven mammalian BCA isozymes spreaded in various tissues differing in intracellular locations. named, BCA I to VII [6]. Crystal structures of human BCA I and II, bovine BCA III, and murine BCA V have been determined [7]. All of these isozymes have the same tertiary structure folding, containing a central 10-stranded beta-sheet as the dominant secondary structure segment [3]. The CA's active site geometry is a composition of a zinc ion which located in a cone-shaped cavity surrounded by three histidyl residues and a solvent molecule [3, 8, 9]. The catalytic mechanism of BCA has been investigated in full detail. It involves the attack of zinc-bound ligand, $OH^-$ to a $CO_2$ molecule bounded to hydrophobic pocket. At the end, metal-coordinated transitional product $HCO_3^-$ ion is replaced by a $H_2O$ [6, 7, 10]. The rate-limiting stage of catalysis is an intramolecular $H^+$ transfer from the metal-bound water molecule to His 64, which acts as a proton carrier between the metal center and buffer solution [7, 11].

Carbonic anhydrase has been extracted from different sources like animal and plant cells, and bacteria like *Neisseria* bacterium [12-15]. Carbonic anhydrase plays a key role in erythrocytes via transportation of $H^+$ and $HCO_3^-$ ions through the cell membranes [16, 17]. In globular proteins particularly the oligomeric ones, the polypeptides rotate around themselves several times, producing numerous contact centers between subunits. The stability of these contact areas is mainly determined by van-der Waals interactions, hydrophobic interactions and hydrogen bonds [18, 19]. The basic concept of a protein structure originates from its secondary structure elements [20-22]. These elements corresponds to protein's stability, at least for locks [21, 22]. The locks can be formed by a variety of combinations of amino acid residues. The detection of these locks may be the most important concern in study of protein structure and folding [22]. The features of interfaces in multi-subunit enzymes and their effect on catalytic activity can be investigated and described with two independent approaches, involving the structural and inactivation kinetics data. Reported that, in alkaline phosphatase from different sources, the results gathered in both methods have reasonable accordance. This theory has been used for a number of enzymes such as alkaline phosphatase [23], superoxide dismutase [24] and amine oxidase [25, 26].

In the present study we applied conformational lock theory for CA from bovine erythrocyte to find additional information on inter-subunit amino acid residues and subunits intermolecular interactions.

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